

Meiotic behavior of the Brazilian table grape cultivar Rubi (*Vitis vinifera*) with a high proportion of seedless berries

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Summary

Meiotic behavior, pollen fertility and germination of the Brazilian table grape cv. Rubi (*Vitis vinifera*) were investigated; this cultivar produced different numbers of seedless berries when cultivated at two different sites: At site A vines produced a high proportion of seedless berries while at site B vines produced berries with a normal number of seeds. Cytological analysis of inflorescences collected from the two sites showed some meiotic abnormalities, the most common being related to chromosomal segregation and telophase micronuclei formation leading to microcyte formation in the tetrads. Cytoplasmic channels, bridges and tripolar spindles were also observed in some microsporocytes. Pollen fertility was high at both sites, approximately 96 % at site A and 98 % at site B. The rate of pollen germination was lower at site A than at site B, suggesting that the absence of seed formation is related to pollen germination rate.

Key words: table grape, *Vitis vinifera*, seedless berries, meiotic abnormalities, unreduced gametes, pollen fertility.

Introduction

Southern Brazil has an ideal climate for viticulture. In the southern State of Rio Grande do Sul many vineyards are dedicated to wine production. However, in the municipality of Marialva in the more northern state of Paraná some seeded table grape cultivars of excellent quality are cultivated. One of these cultivars, cv. Rubi, occasionally presents problems related to berry set, with some bunches having partly seedless berries which impairs commercialization.

Two types of seedlessness are generally recognized in grape varieties, the first arising from parthenocarpy (fruit set without pollination or fertilization), and the second being caused by stenospermocarpy (normal fertilization for fruit set, but ovule abortion occurs during fruit development leaving only partially sclerified seed traces within the berry).

The absence of seeds is genetically controlled in grapes, but despite general progress and the considerable work of breeders for over 70 years, the inheritance of seedlessness is not clearly understood (LEDBETTER and BURGOS 1994; BOU-

QUET and DANGLLOT 1996; ROYTCHEV 1998). Since cv. Rubi is normally seeded, the occurrence of seedless grapes should be investigated on the base of its meiotic behavior.

Seed formation depends on normal meiosis to produce viable gametes. The research reported in this paper investigated the meiotic behavior of cv. Rubi to clarify the seedlessness of some of its berries.

Material and Methods

Inflorescences of cv. Rubi (*Vitis vinifera*) were collected for meiotic studies from vines with different seed production at two different sites in Marialva municipality, Paraná, Brazil. At site A a high percentage of seedless berries was observed in the bunches (Fig. 1), while at site B berries contained a normal number of seeds.



Fig. 1: Grapes collected at site A showing a high number of seedless berries.

Inflorescences were fixed in Carnoy fluid (3:1 ethanol:acetic acid) for 24 h, transferred to 70 % ethanol and stored under refrigeration until needed. At both sites the climatic, edaphic and culture conditions were very similar. Microsporocytes were prepared by the squash technique

and stained with 1 % propionic carmine. Eight inflorescences were scored for each site and more than 1000 cells in each inflorescence were analysed meiotically.

For fertility studies, pollen grains were incubated in 1 % propionic carmine and considered fertile, and while unstained empty ones were noted sterile. For germination studies, pollen grains were incubated in 35 % aqueous sucrose solution at 30 °C for 15 h and then stained with 1 % propionic carmine, a total of 1000 grains being evaluated. In both cases pollen grains were obtained from mature anthers at dehiscence.

The data obtained were statistically analysed by a χ^2 test using a 2x2 contingency table, as recommended by STEEL and TORRIE (1960).

Results

Cytological analyses of inflorescences from both sites showed only a few meiotic abnormalities (Tab. 1), with more than 90 % of the cells presenting normal meiosis. However at site A with more seedless berries some inflorescences had more meiotic abnormalities ($\chi^2 = 550.57$; $P < 0.01$).

The most common meiotic abnormalities were related to chromosome segregation. Precocious chromosome migration to the poles in metaphase I and II (Fig. 2 a, f), non-oriented equatorial plate bivalents (Fig. 2 b) and anaphase I (Fig. 2 c) and II laggards led to micronucleus formation in telophase I (Fig. 2 d), prophase II (Fig. 2 e) and telophase II (Fig. 2 g). As a consequence, microcytes were observed in tetrads (Fig. 2 h) which gave rise to small sterile pollen grains (Fig. 2 h). Some other rare meiotic abnormalities were also

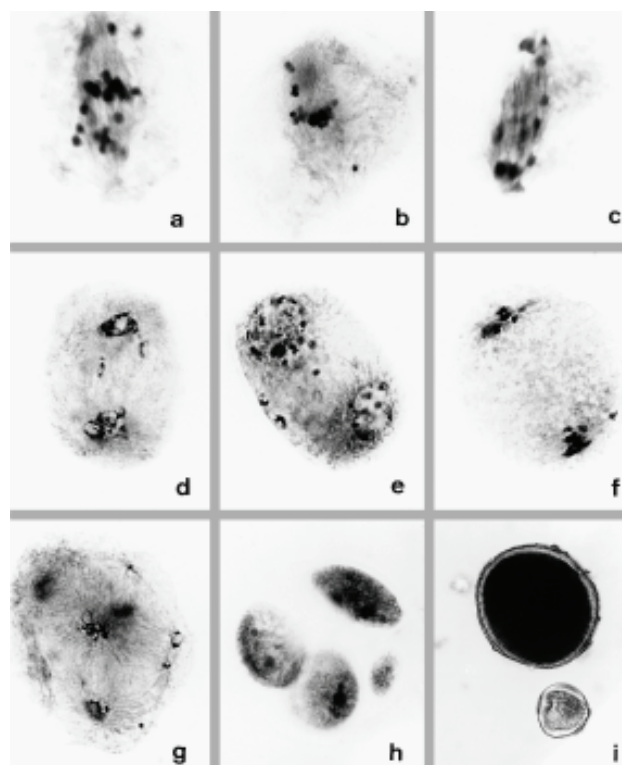


Fig. 2: Meiotic abnormalities related to chromosome segregation. a) Metaphase I showing several chromosomes migrating precociously to the poles. b) Metaphase I with non-oriented equatorial plate bivalents. c) Anaphase I with laggard chromosomes. d) Telophase I with micronuclei. e) Prophase II with micronuclei. f) Metaphase II with precocious chromosome migration in both cells. g) Telophase II with several micronuclei. h) Tetrad with one microcyte. i) Fertile (larger and black) and sterile (small and empty) pollen grains. x 1000.

Table 1

Meiosis in pollen mother cells from inflorescences collected at sites A and B

Site	Inflorescence	Number of cells	Pollen mother cells with normal meiosis (%)							Mean
			M I	A I	T I	P II	M II	A II	T II	
A	1	5502	91.8	67.2	95.6	98.4	95.6	90.1	96.9	92.9
	2	5242	94.8	93.8	98.5	99.4	86.0	69.5	95.8	91.1
	3	9839	90.1	70.8	99.6	100.0	88.8	67.7	96.7	87.7
	4	3950	96.6	87.1	99.8	99.7	90.3	92.6	99.5	95.1
	5	4554	95.3	90.9	95.5	99.6	92.0	96.5	98.6	95.5
	6	3048	98.4	97.0	100.0	99.7	95.1	94.4	98.7	97.6
	7	2979	96.0	99.8	99.3	100.0	94.7	95.9	99.1	97.8
	8	3364	96.3	97.9	100.0	100.0	94.7	98.1	100.0	98.1
Mean			94.9	88.1	98.5	99.6	92.3	88.1	98.2	94.5
B	1	9385	94.5	32.1	99.1	99.7	92.4	90.7	98.9	93.9
	2	8020	97.9	96.7	100.0	100.0	95.8	93.9	98.8	97.6
	3	8586	93.2	82.4	98.6	99.9	94.7	97.2	97.3	94.8
	4	2886	95.5	98.9	100.0	100.0	97.1	100.0	99.7	98.8
	5	3211	97.0	95.5	100.0	99.8	98.9	100.0	99.7	98.7
	6	4344	99.6	99.8	100.0	99.8	99.3	98.6	99.9	99.6
	7	4337	99.5	98.2	99.9	99.4	99.7	96.4	99.9	99.0
	8	5116	99.4	97.3	100.0	99.1	99.7	92.8	99.7	98.4
Mean			96.9	93.8	99.7	99.8	97.2	96.2	99.2	97.6

found, including bridges in anaphase/telophase, cytoplasmic channels (Fig. 3 a) and tripolar spindles in the second division (Fig. 3 b) leading to triad formation, where one microspore was unreduced (Fig. 3 c) resulting in 2n pollen grains (Fig. 3 d).

Pollen fertility was high in inflorescences from both sites (Tab. 2), but at site A sterility was higher ($\chi^2=32.88$; $P < 0.01$). Pollen germination was the character by which seed production at both sites could be differentiated ($\chi^2 = 242.96$; $P < 0.01$), with 78 % germination of site A pollen and 90 % germination of site B pollen.

Discussion

In spite of the economic importance of grapes for some countries, very little effort has been made to study grape cytology because of the small size of grape chromosomes. Meiotic chromosome behavior has been studied by some researchers between 1950 and 1970 (PATIL and PATIL 1996), but in the last two decades few meiotic studies were undertaken, which analysed diploid and tetraploid plants (ME *et al.* 1984) and interspecific hybrids (PATIL and PATIL 1996). Meiotic behavior was slightly abnormal in all the diploid grape varieties analysed, and the abnormalities detected were the same as those found in the cv. Rubi.

The most common meiotic abnormality found in our research as well as in the literature is related to chromosome segregation in metaphase and anaphase, leading to micronuclei formation in telophase, with the first divisions always being more abnormal than the second (SUDHARSAH and SEETHAIAH 1969, 1973; STAUDT and KASSRAWI 1972; ME *et al.* 1984; PATIL and PATIL 1996). These abnormalities have been attributed to univalent chromosomes, precocious chiasma terminalisation at diakinesis or non-oriented equatorial plate bivalents. Anaphase bridges found in some cv. Rubi cells have also been observed by PATIL and PATIL (1996).

The other meiotic irregularity found in cv. Rubi was altered second division spindle polarity which can affect the genetic constitution of the gametes. Second division spindle convergence rejoins at one pole the two sets of non-homologue chromatids segregated in the first division and results in a second division restitution (SDR) nucleus, so-called because the unreduced nucleus was organised after the seg-

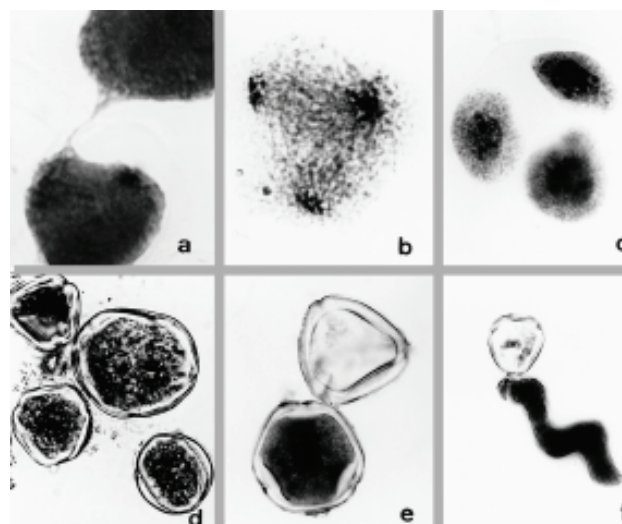


Fig. 3: Rare meiotic abnormalities. a) Microsporocytes connected by cytoplasmic channels. b) Telophase II showing tripolar spindle. c) Triad with one unreduced microspore. d) Unreduced pollen grain. e) Fertile (dark) and sterile (empty) pollen grain. f) Pollen grain germination. x 1000.

regation of homologue chromosomes in anaphase I. The occurrence of unreduced pollen in *Vitis* species has been described by REISCH *et al.* (1988), who analysed over 150 diploid genotypes and found that 4 produced dyads and triads by first division restitution (FDR). Diploid cultivars and tetraploid hybrid cultivars (*V. labruscana* x *V. vinifera*) have also indicated unreduced pollen; cytogenetic studies have shown that the predominant mechanism of unreduced pollen production was FDR accompanied by a lower proportion of co-orientated second division spindles (ZHANG *et al.* 1998). Spontaneous unreduced gametes formed by FDR have also been described by JELENKOVIC and OLMO (1969) in allotetraploid hybrids.

If the character (unreduced gametes) is not genetically controlled, like in potatoes (MENDIBURU and PELOQUIN 1997) and alfalfa (CALDERINI and MARIANI 1997), the occurrence of unreduced gametes is very low and not recurrent. In grapes it is unknown if genes control this character, although the frequency of unreduced gametes is always low. In the diploid and tetraploid cultivars analysed by ZHANG *et al.* (1998), the percentage of unreduced pollen was higher in tetraploids (0.18 - 1.27 %) than in diploids (0.06 - 0.37 %). In cv. Rubi,

Table 2

Pollen fertility and germination for sites A and B

Inflorescence	Pollen fertility (%)		Pollen germination (%)	
	Site A	Site B	Site A	Site B
1	95.5	97.9	80.5	88.8
2	98.0	96.9	75.7	93.3
3	91.9	98.6	74.0	88.5
4	98.4	98.7	79.0	91.5
5	97.9	97.9	81.5	89.8
6	97.8	99.7	76.7	88.5
Mean	96.6	98.3	77.9	90.1

less than 0.5 % of the pollen was unreduced. The formation of unreduced gametes has been investigated in relation to both evolutionary studies (HARLAN and DE WET 1975) and breeding programs (VEILLEUX 1985), however, to be successful with crossings the donor must produce at least 5 % of unreduced gametes.

In cv. Rubi, the low frequency of cells with meiotic abnormalities does not explain the formation of seedless berries, and pollen fertility at both sites being high, even being higher than that reported by SUDHARSAN and SEETHAIAH (1969, 1973) for some Indian grape varieties. The characteristic that best differentiated between sites A and B was the germination ability of pollen. Reduced pollen germination has also been reported by ME *et al.* (1984) for two *V. vinifera* (cv. Barbera) mutants, a tetraploid and a periclinal chimera (2n-4n). Several factors could affect pollen germination, including excess of nitrogen (OKAMOTO and OMORIM 1991), pistil pollen-tube growth inhibitors (OKAMOTO *et al.* 1995), high gibberellic acid concentration (KIMURA *et al.* 1996), boron deficiency (MALAVOLTA *et al.* 1997) and environmental conditions (WANG *et al.* 1996). A more detailed study of the factors initiating or inhibiting pollen germination could explain the causes of the differences between sites A and B.

The absence of seeds may also be caused by ovule anomalies leading to abortion; such anomalies may occur before meiosis because of abnormal development of the nucleus and the integuments or from incomplete anatrophy, or after meiosis because of arrested development of the embryo sac at an early stage or degeneration of the egg in an apparently normal and mature embryo sac (EBADI *et al.* 1996). In grape flower, fertilization of at least one of the 4 ovules is necessary to ensure the development of the pistil into a grape, and imperfect development of the zygote and endosperm may result in seed abortion. VALLANIA *et al.* (1987) reported anomalous ovule development in several seedless grape varieties, but in cv. Barbera mutants the anomalies observed in microsporogenesis did not seem to be sufficient to explain the reduced number of seeds per berry, although ovule development and pollination studies revealed anomalies in embryo sac formation as well as non-fertilization due to difficulties in pollination. Further studies on cv. Rubi may reveal other aspects of the absence of seed formation for this cultivar.

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Received August 21, 2000